 Relation of Optical Coherence Tomography to Microanatomy in Normal and rd Chickens


PURPOSE. To elucidate the relation between optical coherence tomography (OCT) scans and retinal histology in normal and retinal degeneration (rd) chickens.

METHODS. Retinas from adult normal and rd chickens were examined in vivo with OCT at 850 nm and compared quantitatively with stained cryosections of unfixed retinas from the same locations.

RESULTS. The nerve fiber layer (NFL) and inner plexiform layer (IPL) show homogeneous backscatter throughout their thicknesses. NFL reflectivity is ~0.6 log units higher than that of the IPL. The inner nuclear layer shows a low reflectivity; the properties of reflections from ganglion cell and outer nuclear layers are indeterminate. The outer retina and choroid form a large reflective complex. Photoreceptor inner segments produce the highest of these reflections in normal chicken retinas, ~1.5 log units higher than that of the IPL. The retinal pigment epithelium also has a relatively large backscatter coefficient and is the dominant reflector in rd retinas that lack photoreceptors. Choroidal pigment produces an intermediate level of backscatter and is the largest attenuator of signal at 850 nm.

CONCLUSIONS. Quantified OCT signals have a predictable relationship to histology and pathology in chicken retinas. The results from rd retinas represent a first step toward in vivo quantitation of retinal structure in retinal degenerative disease. (Invest Ophthalmol Vis Sci. 1998;39:2405-2416)

CROSS-SECTIONAL RETINAL IMAGING PROVIDES A UNIQUE OPPORTUNITY TO QUANTIFY THE OVERALL THICKNESS OF THE RETINA AND TO IDENTIFY SUBLAMINAR STRUCTURES IN VIVO. IN CLINICAL SETTINGS, THE METHODOLOGY HAS SIGNIFICANT POTENTIAL BOTH AS A DIAGNOSTIC TOOL AND AS A WAY TO MONITOR OBJECTIVELY THE EFFECTS OF THERAPEUTIC INTERVENTION. KEY TO DETERMINING THE VALUE OF THE TECHNIQUES IS AN ACCURATE CORRELATION OF FEATURES OF THE RETINAL SCANS WITH THE UNDERLYING RETINAL ANATOMY. INFORMATION HAS BEEN GAINED FROM THE STUDY OF SCANS FROM PATIENTS WITH RETINAL DISEASES OF KNOWN PATHOLOGY AND DEMONSTRATIONS OF QUALITATIVE RESEMBLANCE OF SCANS TO RETINAL HISTOLOGIC FEATURES. HOWEVER, ONLY A FEW STUDIES HAVE PROVIDED QUANTITATIVE COMPARISONS BETWEEN RETINAL SCANS AND HISTOLOGIC FINDINGS.

To quantify the relationship between cross-sectional retinal images and underlying retinal structures, we used optical coherence tomography (OCT) to scan retinas from normal and rd chickens. OCT results were correlated with retinal histology using unfixed, stained cryosections from the same retinal locations.

MATERIALS AND METHODS

Animals

Eleven adult (10- to 15-month-old) chickens, 8 normal (6 white leghorn, WL; 2 Rhode Island Red, RI) and 3 rd, were used in the study. All chickens were similarly reared on a 12-hour light/dark cycle; experiments were performed under fully light-adapted conditions. The chickens were anesthetized by intramuscular injection of ketamine (12-16 mg/kg) and xylazine (3-4 mg/kg); pupils were dilated with topical vecuronium bromide. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and institutional approval was obtained.

Imaging

Cross-sectional imaging of the retina was performed using an OCT instrument (Humphrey Instruments, San Leandro, CA). The principles of the instrument have been described. In brief, low coherence light (center wavelength = 850 nm, bandwidth = 25 nm) is guided into a Michelson interferometer and split into two light beams directed to the eye and a reference mirror, respectively. The reflections from the two targets, combined at a photodetector, form an interference signal only when the optical path length traveled by the two beams is within the source coherence length (~13 μm in air). The detector current is amplified with a logarithmic amplification circuit and digitized by a 12-bit analog-to-digital converter. The digitized values are proportional to the logarithm of the backscatter from the tissue.

A longitudinal reflectivity profile (LRP) is constructed by rapidly moving the reference mirror (over a depth of 2.7 mm in
were always oriented perpendicular to the long axis of the ONH. OCTs were always oriented perpendicular to the long axis of the ONH using its nasal edge as a guide; the location of each scan was recorded with respect to the superior tip of the ONH. The lateral extent of scans varied from 1.5 mm to 3.5 mm. Most scans were collected as serial sections in groups of 30 with a separation of 150 μm.

**OCT Calibration**

The longitudinal (axial) dimension of LRPs was calibrated using a microscope coverglass (model 0211; Corning Glass, Corning, NY). The glass was placed perpendicular to the scanning beam, and OCT scans were obtained that showed two large peaks corresponding to the specular reflections at the two glass-air interfaces. A calibration factor c (in μm/sample) was calculated by:

\[ c = \frac{I_g \cdot \eta_G / (\Delta n \cdot A)}{n_p \cdot n_G} \]

where \( I_g \) is the thickness of the glass in micrometers; \( n_p \) is the refractive index of the glass (=1.523); \( A \) is the number of samples between the two specular reflection peaks; and \( n_G \) is the group refractive index for the chicken eye (=1.36).\(^{1,2}\)

Lateral dimensions from the OCT instrument, calibrated with respect to an emmetropic human eye, were adjusted for the chicken eye by using the ratio of the retinal magnification factor reported for humans (0.30 mm/visual degree)\(^{16}\) to that of the chicken (0.15 mm/visual degree).\(^{14}\) The validity of this conversion was corroborated experimentally. In an enucleated chicken eye, a metal needle was inserted through the posterior sclera toward the vitreous until the needle was just visible. An OCT scan was determined by measuring the horizontal distance to the dorsal tip of the ONH; this single measure was established using the vertical (along the ONH long axis) distance to the dorsal tip of the ONH, and plateau-like regions, were determined automatically using a computerized algorithm (see Appendix).

**Histology**

The retinas were processed without fixation, dehydration, or embedment to avoid tissue shrinkage or swelling. Animals were euthanatized with sodium pentobarbital immediately after the OCT scan. Eyes were enucleated, and the anterior segments discarded. A retinal region (approximately 10-mm square) centered on the tip of the ONH was excised using a dissecting microscope and then mounted in cryoprotectant (OCT compound; Miles, Elkhart, IN) in a plastic cryostat mold. Gross photographs were taken to document the exact orientation of the retinal sample within the mold; then the preparation was frozen on dry ice.

Serial cryosections were cut at 25 μm in a plane perpendicular to the long axis of the ONH, mounted on chilled glass microscope slides, air-dried, stained with 50% Richardson's methylene blue/azure II mixture, and coverslipped with glycerol. The sections were photographed at 75-μm to 150-μm intervals using Ektachrome ASA160 film and X16 objective (Nikon, Tokyo, Japan). A calibration slide was photographed at the same magnification.

Criteria for section inclusion in the measurements were as follows: no ice crystal damage, section folds, or compression; inner limiting membrane and nerve fiber layer intact and attached; nuclear layers uniform in thickness; photoreceptor oil droplets visible as a continuous layer (normal retinas only); and absence of retinal detachment.

Selected normal and rd retinas were processed for electron microscopy. The retinas were fixed after death in 4% paraformaldehyde and 0.25% glutaraldehyde in 0.13 M phosphate buffer. After fixation for several days, samples of retina were dissected, postfixed in 1% OsO\(_4\) in phosphate buffer, and embedded in Medcast resin. One-micrometer-thick sections were stained with Richardson's mixture for light microscopy, and 90-nm sections were contrasted with uranyl acetate and lead citrate for electron microscopy.

**Image Processing**

Custom computer programs (MATLAB; MathWorks, Natick, MA) were developed for postacquisition image processing of OCT scans and quantitation of histologic sections. Axial motion artifacts originating from micron scale eye and head motion were compensated for by alignment of the LRPs making up each OCT. The alignment was achieved with a dynamic cross-correlation algorithm. A representative LRP (repLRP) was constructed by averaging the first 5 LRPs of each OCT. The cross-correlation function between the repLRP and each successive LRP was used to calculate the shift necessary for alignment. Simultaneously, the repLRP was updated dynamically with LRPs that showed a high cross-correlation coefficient. This approach allowed alignment of OCT scans that entailed slow variations in retinal profile. Alignment was suppressed for scans showing abrupt changes in retinal micromorphology (e.g., Fig. 1A). In regions with uniform retinal layers, neighboring LRPs were averaged to reduce noise that is consistent with speckle originating from low-coherence interferometry. The features of the LRPs, consisting of transitions, peaks, valleys, and plateau-like regions, were determined automatically using a computerized algorithm (see Appendix).

Correspondence between histologic sections and OCT scans was established using the vertical (along the ONH long axis) distance to the dorsal tip of the ONH; this single measure constrained a section and a scan to the same plane because both were obtained perpendicular to the long axis of the ONH. The portion of the histologic section corresponding to the OCT scan was determined by measuring the horizontal (per-
RESULTS
Qualitative Relationship between Cross-Sectional OCT Images and Retinal Histology
Visual inspection of pseudocolor displays of cross-sectional OCT images and the corresponding histologic sections from normal chickens suggests a close relationship between certain scan features and retinal layers (Fig. 1). An OCT image from near the ONH of a WL chicken and the accompanying histologic section illustrate this relationship (Fig. 1A). The high
FIGURE 2. Analysis of optical coherence tomography (OCT) signals originating from the normal inner retina. (A) Unfixed cryosection from the superior tip of the optic nerve head (see schematic, upper right). Longitudinal reflectivity profile (LRP) at the same location is overlaid on inner retinal layers by alignment at the vitreoretinal interface. (B) LRPs obtained at the same location as (A) from four other eyes from white leghorn (WL) and Rhode Island Red (RI) chickens are overlaid on a simplified model of the inner retinal layers. LRP features marked ® represent the three signal transitions (T1, T2, and T3; see Appendix) determined according to the computer algorithm. Depth measurements (α, β) shown are used in (E) and (F). (C) Corresponding LRP overlaid on histology showing thinner nerve fiber layer (NFL) and thicker inner plexiform layer (IPL) at 1-mm superotemporal to the area centralis (schematic). (D) LRPs obtained at the same location as (C) from four other eyes are overlaid on a simplified model. Features and depth measures are similar to (B). (E) Comparison of the OCT depth α and the histologic depth of the sum of NFL and ganglion cell nuclei layer (GCL) in four chickens sampled at various central retinal locations. Equality line is shown as the
laminations parallel retinal and choroidal layers, but exact relationships are not discernible. An even deeper band of low intensity (black) on the right side of the scan disappears on the left; this may represent the termination of the scleral cartilage near the ONH.

OCT and histologic features in the superior retina are compared in a WL and a RI chicken (Figs. 1B, 1C). The rationale for this comparison was to determine the effect of increased ocular pigmentation on OCT images; RI chickens are more highly pigmented than WL. The normal RI chickens also provide a baseline for analysis of the rd chicken, which is a RI strain. The retina of a WL chicken (Fig. 1B) shows two bands of high reflectivity (red and white). The vitreal band corresponds generally to the NFL and the more scleral band to the layers of photoreceptor cells and retinal pigment epithelium (RPE). These two bands are divided by a band of low reflectivity (black, blue, and green), likely corresponding to the inner nuclear layer (INL). The choroid and scleral cartilage have low backscatter with a weak band signaling their interface. The sclera produces intermediate reflectivity (Fig. 1B). The OCT features are generally similar in RI and WL chickens, except that the scleral band is not visible in the RI chicken eyes (Fig. 1C).

**NFL and Inner Retina**

OCT pseudocolor images are formed by a series of LRPs, the logarithm of backscattered light as a function of retinal depth. Inner retinal anatomy and averaged LRPs are compared for two retinal regions that differ in NFL thickness (Figs. 2A, 2C). In the inner retina, the NFL produces the largest backscatter, the inner nuclear layer (INL). The choroid and scleral cartilage have low backscatter with a weak band signaling their interface. The inner retina dependent.

OCT depth (α) from onset of LRP signal (transition 1, T1; see Appendix) to the next abrupt change in signal amplitude (transition 2, T2) is plotted against histologic depth from the vitreoretinal interface through the ganglion cell layer (GCL). An approximate 1:1 relationship is obtained over a range of tissue thicknesses (Fig. 2E). OCT depth (β) to the third transition (T3) shows a close correlation with the histologic thickness of all layers from the vitreoretinal interface through the IPL (Fig. 2F).

A small dark area, approximately 2-mm superonasal to the ONH, is visible on en face fundus images. This region has a thicker GCL and thinner NFL than the surrounding retina (data not shown), and probably represents the afoveate area centralis. Serial OCTs crossing this area from the inferior to superior retina (Fig. 2, see schematic) showed sufficient changes in backscatter to warrant quantitation. The average LRPs demonstrate that the backscatter amplitude of the first band (peak 1, P1; see Appendix) is ~0.6 log units higher than that of the second band (plateau between T2 and T3) throughout the central retina; however, the difference between the two bands drops to ~0.2 log units in the area centralis (Figs. 2G, 2H).

**The Outer Retina in the Normal Chicken**

The outer retina in the normal chicken is composed of the outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor inner segments containing the endoplasmic reticulum-rich myoid and the mitochondria-rich ellipsoids, cone oil droplets, and rod and cone outer segments. In addition, the RPE layer includes microvilli that contain melanin granules and interdigitate with the photoreceptor outer segments and basal cytoplasm that adjoins Bruch’s membrane and contains few melanin granules (Figs. 3A, 3B). One large distal LRP component, hereafter termed outer retina–choroid complex (ORCC), overlays the outer retina and some choroidal structures (Fig. 3A). RI chickens, which have greater choroidal pigmentation, have a broader ORCC than WL chickens (Fig. 3C).

Is it possible to localize the histologic structures that give rise to the ORCC? Onset, offset, and peak locations of the ORCC (Fig. 3C) measured in 21 locations in four chickens are shown as a function of the depth of the histologic layers at the same location (Fig. 3D). The ORCC onset (transition 4, T4; see Appendix) corresponds to the distal ONL and the proximal portion of the photoreceptor inner segments in both strains. The ORCC offset (transition 5, T5) corresponds to the pigmented region of the RPE in WL chickens and to the proximal choroid in RI chickens. The exact location of the ORCC offset in RI chickens is difficult to establish because of the variable collapse of the choroid in these postmortem eyes. The ORCC peak (peak 2, P2), always the largest backscattering amplitude in all OCTs, corresponds mainly to the ellipsoid region of the inner segments, but varies over the region between the myoids and oil droplets (Fig. 3D).

Data from all experiments were summarized by calculating the mean (±1 SEM) distance between ORCC peak and each histologic layer and the distance to ORCC onset and offset (Fig. 3E). The ORCC peak is most approximate to the ellipsoid region and oil droplets, whereas the onset is near to the mean location of the proximal myoid border. The offset in WL chicken retinas corresponds to the proximal RPE and in RI retinas to the proximal choroid (Fig. 3E).
FIGURE 3. Analysis of optical coherence tomography (OCT) signals originating from the normal outer retina. (A) Unfixed cryosection at 1-mm superotemporal to area centralis in a Rhode Island Red (RI) chicken (schematic). The corresponding longitudinal reflectivity profile (LRP) is overlaid. Symbols mark the borders of layers apparent on light micrographs: OPL, outer plexiform layer; ONL, outer nuclear layer; MYOID, myoid region of inner segments; ELLP, ellipsoid region of inner segments; OIL DR, oil droplets of inner segments; OS/AP, outer segments and apical processes of the retinal pigment epithelium; RPE, the pigmented part of the retinal pigment epithelium; CC, choriocapillaris. (B) Electron micrograph of the retina shown in (A) illustrating the outer retinal region. ELM, external limiting membrane. (C) Comparison of LRPs obtained at the same retinal location as (A) in white leghorn (WL) and Rhode Island Red (RI) chickens show the posterior broadening of the outer retina-choroid complex (ORCC). (D) Histology-OCT comparisons at 21 matched locations in two WL and two RI chickens. The borders of histologic...
Figure 4. Optical coherence tomography signals and histology in different severities of rd retinas. (A) Corresponding longitudinal reflectivity profile (LRP) overlaid on the unfixed cryosection nasal to the superior tip of the optic nerve head (see schematic) in a severe phenotype of the retinal degeneration (rd) chicken. (B) Electron micrograph of the outer retina shown in (A), illustrating the degenerate outer retinal region; m, macrophage; M, Muller cells; B, Bruch's membrane; CH, choroid; INL, inner nuclear layer; RPE, retinal pigment epithelium; IS, inner segment; ELM, external limiting membrane. (C) Cryosection and corresponding LRP in a milder rd phenotype at the same retinal location as (A). (D) Electron micrograph of the outer retina shown in (C). ONL, outer nuclear layer; OIL DR, oil droplets of inner segments.

The rd Chicken Retina

Retinal degeneration in the rd chicken is caused by a null mutation in the photoreceptor guanylate cyclase gene. OCTs were correlated with microscopy in rd chickens to determine how a retinal degeneration affecting mainly the outer retina alters the cross-sectional images.
Figure 5. Quantitative analysis of optical coherence tomography (OCT) signals in retinal degeneration (rd) chickens. (A) Unfixed cryosections of rd and normal chickens showing inner retinal layers 1-mm superotemporal to the area centralis (schematic). The corresponding longitudinal reflectivity profiles (LRPs) are overlaid. The signal transitions (transitions 1 and 3, T1 and T3; see Appendix) and depth measurement β are marked as in Figure 2B. (B) Comparison of OCT depth (β) and the histologic depth of the sum of the nerve fiber layer (NFL), ganglion cell nuclei layer (GCL), and inner plexiform layer (IPL) in two rd chickens at several central locations. Equality line is shown; ellipse describes the 95% confidence limits of normal data (shown in Fig. 2F) assuming a bivariate Gaussian distribution. (C) Histology-OCT comparison of the locations of histologic layers and outer retina-choroid complex (ORCC) features (similar to Fig. 3D) in different rd severities (I, II, III) and a representative normal retina. OPL, outer plexiform layer; ONL, outer nuclear layer; MYOID, myoid region of inner segments; ELLP, ellipsoid region of inner segments; OIL, oil droplets of inner segments; OS/AP, outer segments and apical processes; RPE, retinal pigment epithelium; CC, choriocapillaris. (D) The reflectivity of the ORCC peak relative to the plateau region between the second and third transitions (T2 and T3; see Appendix) in rd with different severities; normal (± 1 SD) reflectivity shown with dashed lines. (E) ORCC from an rd retina with a severity of III is overlaid with one from a normal retina; waveforms are aligned by corresponding histology. ORCC peak (peak
The rd retinas showed variable disease severity. The most severe phenotype (Figs. 4A, 4B) lacked photoreceptors, but had intact inner retinal layers (NFL, IPL, and INL). The proximal components of the LRP show abrupt changes in waveform corresponding to the inner retinal histologic layers. The ORCC spans a region including RPE and choroid (Fig. 4A). In a less severe phenotype (Fig. 4C), the OPL and ONL are retained, although abnormally thinned (Fig. 4D). All rods are lost, but some cone inner segments and tiny outer segments are retained (Fig. 4D). The proximal LRP again shows abrupt changes in waveform corresponding to the inner retinal histology. The ORCC overlays the degenerate photoreceptor layer and spans the RPE and choroid.

Figure 5 quantifies the relationship between OCT and retinal histology in the rd chickens. The IPL and INL at a location superior to the area centralis appear thinner compared to a normal RI retina (Fig. 5A). In the rd chicken, like the normal chicken, OCT depth (β) from onset of LRP to the third transition (T3; Appendix) closely correlates with the histologic depth from the vitreoretinal interface through the IPL, but it tends to be thinner than normal by ~25 µm (Fig. 5B).

Layer-by-layer quantitation and comparison of histology and ORCC are shown for three different degrees of severity of retinal degeneration (Fig. 5C). Onset (T4; Appendix) to offset (transition 5, T5) of ORCC in the most severe (III) and intermediate (II) phenotypes corresponds to RPE and choroid. In the mildest phenotype (I), ORCC spans the degenerate photoreceptor layer into the choroid. The ORCC peak (peak 2, P2) is located near the RPE in more severely degenerated retinas, whereas it is near the myoid in the less severe phenotypes. Reflectivity of the ORCC peak (relative to the plateau between T2 and T3) in rd chickens is ~0.5 log units lower than normal chickens (Fig. 5D). An ORCC from an rd chicken with severity III overlaid on the ORCC of a normal chicken illustrates the differences in the two waveforms (Fig. 5E). The waveforms have been aligned by matching histology, specifically the location of the pigmented RPE. The comparison suggests that reflections from the photoreceptor inner segments and the RPE contribute to the normal ORCC; in the absence of photoreceptors, the RPE becomes the dominant reflector (Fig. 5E).

The contribution of choroid to the ORCC is analyzed in scans from two adjacent regions of the rd retina. Notable by funduscopy in rd chickens are peripapillary and peripheral retinal areas with apparently less choroidal pigmentation. Histology of these areas shows breaches in the cartilaginous layer and reduced choroid (Fig. 5F); normal chickens show similar gaps in the cartilage but with a preserved choroid. Overlaid LRP from areas with and without visible choroid indicate that the choroid contributes a backscattering signal to the distal ORCC and attenuates deeper reflections (Fig. 5G).

**DISCUSSION**

The reflectance properties of the ocular fundus have been measured noninvasively using a variety of methods. OCT is a new interferometric technique that maps the reflectivity of the fundus cross-sectionally and with high lateral and longitudinal resolutions. OCT has the potential to provide quantitative information about retinal and choroidal structures in vivo. By visual inspection, OCT scans appear laminated, suggesting that different retinal layers may be recognizable by their reflectivity “signature” as the probe beam traverses the tissue. However, the exact relationship between reflectivity and retinal layers is not fully understood and prompted the studies reported here. We correlated OCT reflectivity with histology, both obtained at the same retinal location. Experiments were performed in normal chickens with different levels of ocular pigmentation and in rd chickens, now recognized as an animal model for Leber congenital amaurosis. We did not use conventional tissue processing procedures that use aldehyde fixation, alcohol dehydration, and embedment, all of which cause variable swelling and/or shrinkage of the retina. Instead, we used cryosections of unfixed retinas that had not been dehydrated or embedded to preserve tissue dimensions most accurately. Although we cannot rule out a small amount of compression during sectioning, the tissues appeared intact with no notable distortion of the retinal layers, and the steps known to produce alterations in dimensions were avoided.

**Backscattering Properties of the Inner Retina**

There was a distinct layer of high reflectivity immediately scleral to the vitreoretinal interface. The end of this layer was marked by a transition to a lower reflectivity occurring at or near the GCL. Variation in depth of the high reflectivity layer corresponded to the variable thickness of the NFL (+GCL) (Fig. 2E). These findings are similar to those from other studies using interferometric and histologic methods in chicks, monkeys, and humans. The component of the OCT waveform originating primarily from the NFL changed in shape as a function of the thickness of this layer. In regions of the retina with a thick NFL, the signal approximated a square wave (Fig. 2A). This suggested that a constant amount of backscatter was originating from throughout the layer. With decreasing thickness of the NFL, the waveform narrowed but retained signal amplitude (Fig. 2C). When the NFL decreased to the level of the depth resolution of the instrument, there was a clear decline in signal originating from this layer, most evident in the area centralis (Figs. 2G, 2H). The observed changes in the reflectivity profile of the NFL were consistent with the convolution of the depth transfer function with a homogeneous backscattering layer of varying thickness. How much variability in reflectivity was introduced by the well-known directional reflectance of the NFL is uncertain, but the small pupil of the chicken limited the range of imaging geometry.

The inner limiting membrane, thought to produce a highly directional reflection, did not appear to contribute to the OCT scan in the chicken retina. This conclusion is drawn from the absence of a large transient reflection in areas with a thick or very thin NFL. In theory, such an inner limiting membrane-
Backscattering from the Outer Retina

The largest and most complex OCT backscattering signal, the ORCC, in the chicken originates from multiple layers in the distal retina through the proximal chorioid. To determine more exact signal origins, we compared waveform features with histologic landmarks. In addition, the complex was analyzed by studying differences in pigmentation in normal chickens and loss of certain layers due to inherited retinal degeneration.

In normal WL and RI chickens, the onset and peak of the ORCC overlay the photoreceptor layer. Specifically, the onset corresponded to the proximal inner segment and the peak to the distal inner segment. The mitochondria of the ellipsoid may increase the local refractive index and may represent a source of backscatter. Alternative or additional contributors to backscatter in this region are the oil droplets, which may be derived from mitochondria. Localization of the peak close to the ellipsoid region of the inner segments differs from previous literature, which assigned the major reflector to more distal structures in chickens and primates. The basis for differences in results is not known but may be due to dissimilar methods, especially in the preparation of retinal tissues for microscopy.

Backscatter distal to the ORCC peak in the WL chickens extended into the region of the RPE (Fig. 3E); there was no backscatter in the choroid, but signals originating from the sclera were detectable (Fig. 1B). In the RI chickens, the ORCC extended past the choriocapillaris into the proximal chorioid (Fig. 3E), and there were no deeper signals (Fig. 1C). We attribute the differences in OCT results in the animals to the basis of the signals.

Implications for OCT in Humans

The present study not only confirmed some intuitive notions about the relationship between OCT signals and retinal microanatomy but also provided some unexpected data, especially for the outer retina. In humans, the major OCT reflection (corresponding to what has been called ORCC in the current work) has been suggested to originate from the RPE and choriocapillaris. This conclusion is based mainly on comparisons of normal retinal scans with those from diseased retinas, specifically RPE detachments and neural retinal detachments. Some investigators have hypothesized that reflections from photoreceptor outer segments may contribute to the major reflection in OCT in humans consistent with what we found in the chicken. Caution is warranted, however, in extrapolation of our results from the avian to the human eye. There are significant differences between the two species. For example, the avian eyes have a deeper IPL, a narrower ONL, oil droplets, double cones, lower rod-to-cone ratios, photomechanical abilities including contractile activity of myoids, and migration of pigment in and out of the processes of RPE. How the OCT results in the two strains of chickens we studied with different ocular pigmentation relate to humans who differ in ocular pigmentation awaits further study.

Considering that OCT is being used increasingly to investigate, diagnose, and monitor disease in the retinas of humans, insights gained in the present study may direct further studies in mammalian and human retinas to elucidate the basis of the signals.

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References

OCT-Histopathology Correlation in Chickens


APPENDIX

Automated Feature Detection

Averaged LRP s had for the most part similar features consisting of transitions, peaks, valleys, and plateau-like regions (see Fig. A1). A computer algorithm based on signal amplitude and slope was used to locate reproducibly and automatically the signal features. Signal baseline is the mean value of the first (vitreal) 30 sample amplitudes. Signal start (S) is the first (vitreal) location where 10 consecutive samples are 0.1 log units greater than mean baseline amplitude. Transition 1 (T1) is the...
first maximum of the slope encountered on the scleral side of signal start (S). Peak 1 (P1) is the first zero-crossing of the slope scleral to T1. Peak 2 (P2) is the maximum amplitude point scleral to P1, but P2 is constrained to be more than 10 samples away from P1. Valley (V) is the minimum amplitude point between P1 and P2. Transition 2 (T2) is the minimum slope within the vitreal half of the distance between P1 and V. Transition 3 (T3) is the minimum slope within the scleral half of the distance between P1 and V. Transition 4 (T4) is the maximum slope between V and P2. Signal end (E) is the first point to reach the same signal amplitude as S scleral to P2. Transition 5 (T5) is the first minimum of the slope function smaller than $-0.07$ log units/pixel encountered when searching from E to P2.